

We claim:

1. A recombinant thermostable DNA polymerase which is characterized in that
  - a) in its native form said polymerase comprises the amino acid sequence  
LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4,  
5 6, 9, and 10 of said sequence are any amino acid residue, and "Xaa" at position 7 of said  
sequence is Val or Ile;
  - b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and
  - c) said thermostable DNA polymerase has a level of discrimination against incorporation  
10 of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.
2. The recombinant thermostable DNA polymerase of claim 1 wherein said nucleotide is a  
dideoxynucleotide and said level of discrimination is at least 3-fold lower than that of said native  
form of said polymerase.
3. The recombinant thermostable DNA polymerase of claim 2 wherein said level of  
discrimination is measured by determining the concentration of a dideoxynucleotide labeled with  
a fluorescein dye that is required for 50% inhibition of DNA synthesis.
- 20 ~~4. The thermostable DNA polymerase of claim 2 wherein said polymerase is from a  
thermophilic species selected from the group consisting of *Thermosipho africanus*, *Bacillus  
caldotenax*, and *Bacillus stearothermophilus*.~~
- 25 5. The thermostable DNA polymerase of claim 2 wherein said polymerase is from a *Thermus*  
species.

6. The recombinant thermostable DNA polymerase of claim 5 which is characterized in that  
a) in its native form said polymerase comprises the amino acid sequence  
LeuSerXaaXaaLeuXaalleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or  
Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and

c) said thermostable DNA polymerase has a level of discrimination against incorporation  
of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.

7. The recombinant thermostable DNA polymerase of claim 6 which is characterized in that  
a) in its native form said polymerase comprises the amino acid sequence  
LeuSerGlnXaaLeuAlalleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any  
amino acid

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and

c) said thermostable DNA polymerase has a level of discrimination against incorporation  
of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.

8. The recombinant thermostable DNA polymerase of claim 7 which is characterized in that  
said "Xaa" at position 4 is mutated to Lys.

9. The thermostable DNA polymerase of claim 2 wherein said polymerase is from a  
*Thermotoga* species.

10. The recombinant thermostable DNA polymerase of claim 9 which is characterized in that  
a) in its native form said polymerase comprises the amino acid sequence  
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any  
amino acid and "Xaa" at position 7 is Val or Ile.

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b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu; and

c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
5 form of said polymerase.

11. A nucleic acid sequence encoding a recombinant thermostable DNA polymerase which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence

10 LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6, 9, and 10 of said sequence are any amino acid residue, and "Xaa" at position 7 of said sequence is Val or Ile;

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that "Xaa" at position 4 is not mutated to Glu; and

15 c) said thermostable DNA polymerase has a level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native form of said polymerase.

20 12. The nucleic acid sequence of Claim 11 wherein said nucleotide is a dideoxynucleotide and said level of discrimination is at least 3-fold lower than that of said native form of said polymerase.

25 13. The nucleic acid sequence of claim 12 wherein said level of discrimination is measured by determining the concentration of a dideoxynucleotide labeled with a fluorescein dye that is required for 50% inhibition of DNA synthesis.

30 14. The nucleic acid sequence of claim 12 wherein said polymerase is from a thermophilic species selected from the group consisting of *Thermosipho africanus*, *Bacillus caldotenax*, and *Bacillus stearothermophilus*.

15. The nucleic acid sequence of claim 12 wherein said polymerase is from a *Thermus* species.

16. The nucleic acid sequence of claim 15 which is characterized in that

5 a) in its native form said polymerase comprises the amino acid sequence  
LeuSerXaaXaaLeuXaaIleProTyrGluGlu (SEQ ID NO: 2), whereby "Xaa" at position 3 is Gln or  
Gly, "Xaa" at position 4 is any amino acid, and "Xaa" at position 6 is Ser or Ala

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and

10 c) said thermostable DNA polymerase has a level of discrimination against incorporation  
of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.

17. The nucleic acid sequence of claim 15 which is characterized in that

15 a) in its native form said polymerase comprises the amino acid sequence  
LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any  
amino acid

b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and

20 c) said thermostable DNA polymerase has a level of discrimination against incorporation  
of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.

18. The nucleic acid sequence of claim 17 which is characterized in that said "Xaa" at  
25 position 4 is mutated to Lys.

19. The nucleic acid sequence of claim 12 wherein said polymerase is from a *Thermotoga*  
species.

20. The nucleic acid sequence of claim 19 which is characterized in that

a) in its native form said polymerase comprises the amino acid sequence  
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is  
any amino acid and "Xaa" at position 7 is Val or Ile.

5 b) said "Xaa" at position 4 is mutated in comparison to said native sequence, except that  
"Xaa" at position 4 is not mutated to Glu; and

c) said thermostable DNA polymerase has a level of discrimination  
against incorporation of nucleotides labeled with fluorescein family dyes which is reduced in  
comparison to the native form of said polymerase.

10 21. A method of DNA sequencing which comprises

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence  
LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9,  
and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino  
15 acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile

ii) said polymerase has a reduced level of discrimination against incorporation of  
nucleotides labeled with fluorescein family dyes, and

b) providing a dye-terminator labeled with a negatively charged fluorescent dye, and

c) performing a dye-terminator sequencing reaction.

20 22. The method of claim 21 wherein said nucleotide is a dideoxynucleotide and said level of  
discrimination is measured by determining the ratio of the concentration of a dideoxynucleotide  
labeled with a fluorescein dye required for 50% inhibition of DNA synthesis versus the  
concentration of an unlabeled dideoxynucleotide required for 50% inhibition.

25 23. The method of claim 22 wherein said ratio is 4 or less.

24. The method of claim 22 wherein said polymerase is from a thermophilic species selected  
from the group consisting of *Thermosipho africanus*, *Bacillus caldotenax*, and *Bacillus*  
*stearothermophilus*.

25. The method of claim 22 wherein said thermostable DNA polymerase is from a *Thermus* species.

26. The method of claim 25 wherein said amino acid sequence comprises:

5 LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

27. The method of claim 26 wherein said "Xaa" at position 4 is Lys.

10 28. The method of claim 22 wherein said polymerase is from a *Thermotoga* species.

29. The method of claim 28 wherein said amino acid sequence comprises:

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

30. The method of claim 29 wherein said "Xaa" at position 4 is Arg.

31. A method of producing labeled DNA which comprises:

a) providing a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile.

ii) said polymerase has a reduced level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes;

25 b) providing a nucleotide labeled with a fluorescein family dye, and

c) performing a DNA synthesis reaction.

32. A method of producing labeled primer extension products which comprises:

a) providing a thermostable DNA polymerase characterized in that

30 i) said polymerase comprises the amino acid sequence

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile.

ii) said polymerase has a reduced level of discrimination against incorporation of nucleotides labeled with fluorescein family dyes;

5       iii) said polymerase also comprises the second amino acid sequence SQIXLR(V/I) (SEQ ID NO: 18) where "X" is any amino acid except E,

iv) said polymerase has reduced discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes;

b) providing a ribonucleotide labeled with a fluorescein family dye, and

10       c) performing a primer extension reaction.

33. A kit for DNA sequencing which comprises

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

15       LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile

ii) said polymerase has reduced discrimination against incorporation of nucleotides labeled with fluorescein family dyes, and

20       b) a terminator labeled with negatively-charged fluorescent dye.

34. The kit of claim 33 wherein said reduced level of discrimination is measured by determining the ratio of the concentration of ddNTP labeled with a fluorescein family dye required for 50% inhibition of DNA synthesis compared to that for an unlabeled ddNTP and said  
25       ratio is 4 or less.

35. The kit of claim 34 wherein said amino acid sequence comprises:

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

30       36. Kit of claim 35 wherein said "Xaa" at position 4 is Lys.

37. The kit of claim 34 wherein said amino acid sequence comprises:  
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any  
amino acid except Glu and "Xaa" at position 7 is Val or Ile.

38. Kit of claim 37 wherein said "Xaa" at position 4 is Arg.

39. A kit for DNA sequencing which comprises

a) a mutant thermostable DNA polymerase characterized in that

i) in its native form said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 4, 6,  
9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 7 of this sequence  
is Val or Ile

ii) said amino acid sequence is mutated, except that "Xaa" at position 4 is not mutated to  
Glu; and

iii) said thermostable DNA polymerase has a level of discrimination against incorporation  
of nucleotides labeled with fluorescein family dyes which is reduced in comparison to the native  
form of said polymerase.

40. The kit of claim 39 wherein said level of discrimination is at least 5-fold lower than that  
of said native form of said polymerase.

41. The kit of claim 40 wherein said recombinant thermostable DNA polymerase is  
characterized in that in its native form said polymerase comprises the amino acid sequence

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any  
amino acid.

42. Kit of claim 41 wherein said "Xaa" is mutated to Lys.

43. The kit of claim 40 wherein said recombinant thermostable DNA polymerase is  
characterized in that in its native form said polymerase comprises the amino acid sequence



LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid and "Xaa" at position 7 is Val or Ile.

44. Kit of claim 43 wherein said "Xaa" is Arg.

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45. A kit for producing labeled DNA which comprises

a) a thermostable DNA polymerase characterized in that

i) said polymerase comprises the amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 7), whereby "Xaa" at positions 3, 6, 9, and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile

ii) said polymerase has reduced discrimination against incorporation of nucleotides labeled with fluorescein family dyes, and

b) a nucleotide labeled with a negatively-charged fluorescent dye.

46. The kit of claim 45 wherein said amino acid sequence comprises:

LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:14), whereby "Xaa" at position 4 is any amino acid except Glu.

47. Kit of claim 45 wherein said "Xaa" at position 4 is Lys.

48. The kit of claim 45 wherein said amino acid sequence comprises:

LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 15), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

49. Kit of claim 48 wherein said "Xaa" at position 4 is Arg.

50. A kit for producing labeled primer extension products which comprises

a) a thermostable DNA polymerase which is characterized in that

i) in its native form, the polymerase comprises the first amino acid sequence

LeuSerXaaXaaLeuXaaXaaProXaaXaaGlu (SEQ ID NO: 1), whereby "Xaa" at positions 3, 6, 9,

and 10 of this sequence are any amino acid residue, and "Xaa" at position 4 can be any amino acid except Glu, and "Xaa" at position 7 of this sequence is Val or Ile;

ii) the polymerase has reduced discrimination against incorporation of nucleotides labeled with fluorescein family dyes;

5 iii) the polymerase also comprises the second amino acid sequence SQIXLR(V/I) where "X" is any amino acid except;

iv) the polymerase has reduced discrimination against incorporation of ribonucleotides labeled with fluorescein family dyes; and

b) a ribonucleotide labeled with a fluorescein family dye.

10 51. The kit of claim 50 wherein said amino acid sequence comprises:  
LeuSerGlnXaaLeuAlaIleProTyrGluGlu (SEQ ID NO:3), whereby "Xaa" at position 4 is any amino acid except Glu.

15 52. Kit of claim 51 wherein said "Xaa" at position 4 is Lys.

20 53. The kit of claim 50 wherein said amino acid sequence comprises:  
LeuSerValXaaLeuGlyXaaProValLysGlu (SEQ ID NO: 4), whereby "Xaa" at position 4 is any amino acid except Glu and "Xaa" at position 7 is Val or Ile.

54. Kit of claim 53 wherein said "Xaa" at position 4 is Arg.